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The relevance of antigen binding to the pathogenicity of lupus autoantibodies

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Although lupus autoantibodies provide diagnostic value, discordance between serum levels and nephritis poses mechanistic questions. Krishnan and co-workers report that only those that crossreact with basement membrane components produce immune deposits. Thus, other glomerular binding properties probably define where deposits form. Thereafter, Fc- and complement-mediated events influence disease expression. Clearly other factors determine the ultimate phenotype; however, the findings provide insights into the variable disease patterns in lupus nephritis.

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The discovery that circulating anti-DNA antibodies are a diagnostic feature of patients with systemic lupus erythematosus helped define the disease (reviewed by Foster *et al.*[1]). Antibodies against double-stranded DNA are relatively specific, and the presence of other autoantibodies (for example, anti-SmRNP) provides further diagnostic support. Initial correlation of serum autoantibody levels with disease activity in some patients led to the notion that these antibodies were pathogenic, and this was supported by the finding that high-affinity, anti-DNA antibodies were present in the kidney eluates of lupus patients. However, it turned out that these and other correlations were inconsistent and of limited clinical utility in predicting either the type of nephritis or disease severity in individual patients.[2] In this regard, distinguishing pathogenic from non-pathogenic antibodies has been difficult, and interpretation is further muddled by diverse clinical manifestations among patients. Not only does organ involvement vary, but during the course of a person's lifetime, organs can be involved variably and differentially.[3] In one sense, the kidney represents a microcosm of systemic manifestations, with a varying spectrum of lesions involving glomeruli, larger vessels, and tubules. Furthermore, the spectrum of glomerular involvement is considerable, from only immune deposits without pathologic or clinical evidence of disease, to crescentic glomerulonephritis, accompanied by severe interstitial and vascular inflammation, with rapidly progressive renal failure. Nevertheless, in human lupus, the quantity and location of glomerular immune deposits generally correlate with the class and severity of nephritis.[1,3] Many factors contribute to pathogenesis; however, immune deposit formation is critical. Therefore, what makes lupus autoantibodies form immune deposits is crucial to deciphering the disease process.

Keys to understanding autoantibody pathogenicity have been, in part, derived from analysis of inbred murine strains that spontaneously develop lupus. Some strains develop severe nephritis, whereas others do not. Comparison of serum antibodies among lupus strains, along with analysis of IgG eluted from the kidneys of nephritic mice, revealed that the eluted antibodies were more broadly reactive than the serum antibodies, in that they reacted and crossreacted with multiple autoantigens, including cell-surface, matrix, and basement membrane antigens.[1,4] By contrast, serum autoantibodies were more restricted with specificity directed at DNA and nucleoproteins, and they were not crossreactive. Comparisons in human lupus provided similar findings.[5] A partial explanation for this crossreactivity was that the seemingly diverse antigens shared epitopes, although induced fit may also play a role. Although this does not explain autoantibodies with reactivity to glomerular antigens, per se, collectively the findings suggest that antigen binding is relevant to pathogenesis in general, and immune deposit formation in particular.

Evaluation of the pathogenicity of monoclonal autoantibodies derived from lupus-prone mice and lupus patients provided additional insights. After injection into normal mice, some anti-DNA antibodies produced immune deposits and nephritis, whereas others did not.[6,7] The pathogenic antibodies were typically IgG and more crossreactive, much like those eluted from the nephritic kidneys. It is particularly noteworthy that, among the pathogenic subset, the location of immune deposits formed varied with the individual antibodies. Strikingly, this was especially apparent when antibodies from different laboratories were compared. For example, some formed predominantly subendothelial deposits, whereas others formed mesangial deposits, and others were more like cryoglobulins. Furthermore, independent analysis revealed that lupus autoantibodies that shared encoding V gene sequences, derived from different mice and different strains, produced similar pathologies.[8–10] Moreover, autoantibodies derived from lupus-prone mice that were encoded by sequences similar to those encoding pathogenic anti-DNA antibodies, but that did not bind to DNA, bound to glomerular autoantigens (for example, laminin) and produced pathologies similar to those produced by

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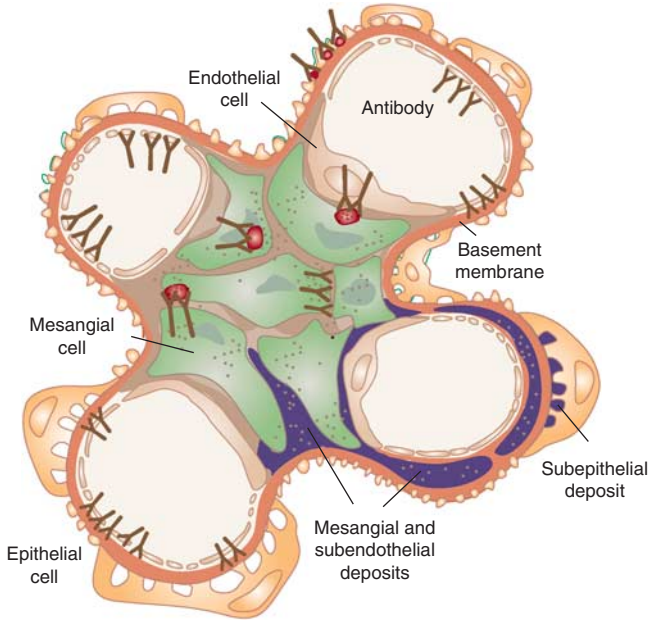

Figure 1 | Immune deposit formation in systemic lupus erythematosus. Antibodies (Y-like stick figures) bind to the glomerular basement membrane (GBM) or glomerular cells to initiate immune deposit formation. Krishnan and co-workers[13] demonstrate autoantibody binding to GBM constituents, whereas others have demonstrated binding to glomerular cells per se. These local interactions initiate FcR engagement and complement activation that, in turn, lead to inflammation, proteinuria, and/or reduced glomerular filtration rate. Soluble circulating immune complexes engage FcR on glomerular cells (for example, mesangial, center of figure), leading to further cellular activation, amplifying disease. The antigenic specificity of autoantibodies against GBM and cell-surface antigens determines the location of immune deposits, and the quantity and site of immune deposits influence phenotypic expression and severity of disease. Variability in antigen specificities and IgG isotype of pathogenic antibodies among individuals contributes to differences in disease expression.

the nephritogenic anti-DNA antibodies.[1] Collectively the findings suggested that specific antigen-binding properties of the autoantibodies mediated immune deposit formation by direct binding to glomerular antigens, and that these specificities influenced both the location of immune deposits formed and subsequent pathology (Figure 1).

Other mechanisms of immune deposit formation have been proposed.[11,12] *In situ* immune deposit formation of nucleosomes and autoantibodies has been suggested to occur by initial charge–charge interaction of positively charged nucleosomes with negatively charged constituents in the basement membrane (for example, heparan sulfate), with subsequent binding of either anti-DNA antibodies or anti-nucleosome antibodies to antigenic components within the exposed nucleosome (for example, DNA). Immune deposit formation in this manner seems feasible; however, overt nephritis by this mechanism has not been demonstrated.

Krishnan and co-workers[13] (this issue) further address the relevance of these *in situ* mechanisms by comparing the immune deposit-forming capacity of related anti-DNA antibodies, but with different relative affinities for basement membrane constituents. They find that only anti-DNA antibodies that bind to components of the basement membrane form immune deposits, activate complement, and induce proteinuria. Furthermore, they observe the presence of autoantibodies alone within the deposits, without other chromatin material, further supporting the direct binding mechanism. This is consistent with previous observations demonstrating pathogenicity of lupus autoantibodies via direct interactions with glomerular antigens. They clearly define this as a major mechanism of immune deposit formation in lupus nephritis.

However, this is probably the tip of the iceberg in terms of disease-relevant antigens in lupus nephritis. Seek, and ye shall find. Many antigenic specificities for autoantibodies have been observed in individuals with lupus, and these specificities contribute to disease variability. Anti-DNA antibodies represent a significant fraction of deposited immunoglobulins, but the larger fraction of immunoglobulins eluted from nephritic kidneys do not bind to DNA.[4,5,14] In this regard, we observed that monoclonal lupus autoantibodies with glomerular binding properties (for example, versus laminin) are encoded by genes encoding anti-DNA antibodies, suggesting that they have common origins. Importantly, the results indicate that other direct binding activities are relevant to pathogenesis.[15] The findings also indicate that in a given person with lupus, the dominant autoantigen-binding profile, at a given point in time, determines whether and where immune deposits will form. This probably explains the relative lack of correlation of either anti-DNA levels or circulating immune complex levels with disease activity. The phenomena also contribute to the diversity of lesions observed among patients.

The *in situ* mechanism involving so-called 'planted antigens' in the kidney, with subsequent autoantibody binding, probably amplifies disease, as with binding of anti-nucleosome antibodies to nucleosomes or of anti-IgG to deposited IgG (that is, by rheumatoid factors). In this context, antibody binding to fixed glomerular antigens, *in situ*, provides an ideal scaffold for subsequent FcR engagement and local complement activation that is necessary for amplification of inflammation.[16]

The results of Krishnan *et al.*[13] clarify previous discrepancies. Local immune deposit formation has been observed to be critical for initiating nephritis in other experimental models and human disease. Preformed, circulating immune complexes do not appear to play a major role in this context. This conclusion is in

accord with clinical observations that dissociate immune complex levels from disease severity. Furthermore, it is consistent with similar mechanisms in other antibody-dependent experimental autoimmune models and human nephritis, where disease is dependent on either local immune complex formation or antigen-specific cellular responses. In lupus, antigenic specificity is essential, and direct binding of autoantibodies is key to pathogenesis. This principle helps explain variable organ involvement in lupus patients. Thus, although systemic lupus erythematosus is characterized by multiorgan involvement and the presence of circulating anti-DNA antibodies, the autoantigens that autoantibodies react with are crucial to where deposits form. This defines organ involvement in an individual patient. In the context of nephritis, the capacity of autoantibodies to bind to glomerular antigens determines and influences the quality and intensity of disease activity. In one sense, the kidney represents a microcosm of the organism, with variable disease depending on the antigenic specificity in a given lupus patient. Whereas anti-erythrocyte antibodies lead to hemolytic anemia, antineuronal antibodies produce cerebritis, and autoantibodies that react with renal antigens initiate nephritis of one type and/or another.

Once immune deposits form, the capacity of the deposited antibodies to engage FcR and activate complement influences disease severity. Thereafter, other mechanisms may be operative in amplifying immune deposit formation, with loss of tolerance via neoantigen exposure, binding of anti-IgG, and so on. Nevertheless, the results of Krishnan and co-workers[13] provide conclusive evidence that the initial events are mediated by autoantibody binding to glomerular antigens. They demonstrate this for anti-DNA antibodies. In this context it is likely that other autoantibodies/autoantigens are involved in the process. Although anti-DNA antibodies constitute a significant fraction of deposited IgG, at most, they represent less than half of the deposited IgG. Individual observations of autoantibody binding to various renal antigens contribute, and these specificities influence the variable disease process. The particular specificity that dominates in a given individual probably affects disease expression.

Is that all there is? Definitely not. Both B cells and T cells themselves actively participate in various stages of disease, and macrophages play a major role. The intensity of the inflammatory and fibrogenic responses is crucial to severity, and this is determined by the autoimmune response, the systemic inflammatory response, and the kidney's response to the assault. Nevertheless, in addition to clarifying the major mechanism of immune deposit formation in lupus nephritis, the results of Krishnan and co-workers[13] raise questions pertaining to the functional consequences of antigen ligation, per se, during the disease process. Does ligation interfere with properties associated with filtration? With normal cell–cell interactions? With cell–matrix interactions? With normal repair processes and events? A more precise understanding of the participants and mechanisms involved in the disease process should lead to better means to monitor disease activity.

If confirmed in human lupus, the results have implications for both biomarker development and therapy. More refined biomarkers using glomerular cell-surface antigens and glomerular matrix proteins have the potential to define when the kidney-specific, autoimmune response is active. Timing immunosuppressive therapy to this window in patients with nephritis would help define when to initiate immunosuppressive therapy and when to limit its use. This would be especially helpful to improve reversibility, reduce fibrosis, and limit the toxic effects of immunosuppressive therapy. Additionally, strategies might be devised that either eliminate or silence these particular B cells, limit autoreactive B cell–T cell interactions, or prevent nephritogenic antibodies from depositing. Although targeting anti-DNA antibodies was not successful, targeting either kidney-specific autoantibodies or the specific population of T cells that activate them has the potential to limit disease with less toxicity. Validation of the findings in human lupus should be particularly helpful.

DISCLSOURE

The author declared no competing interests.

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